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PATENT

I hereby certify that on the date specified below, this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to the Commissioner for Patents, Washington, DC 20231.

Date

Millicent A. Scarlett

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Martin A. Cheever and Mary L. Disis
Application No.	:	09/088,951
Filed	:	June 2, 1998
For	:	METHODS AND COMPOSITIONS TO GENERATE IMMUNITY IN HUMANS AGAINST SELF TUMOR ANTIGENS BY IMMUNIZATION WITH HOMOLOGOUS FOREIGN PROTEINS
Examiner	:	Karen A. Canella, Ph.D.
Art Unit	:	1642
Docket No.	:	920010.535

#28
KD
4-30-02

Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner:

We, Martin A. Cheever and Mary L. Disis, hereby declare as follows:

1. We are co-inventors of the subject matter described in the above-identified patent application (hereinafter referred to as "subject application").

2. All of the work described within this Declaration was performed in the United States, by ourselves or on our behalf and under our direction.

3. We have reviewed our laboratory records, including the Exhibit submitted herewith, and readily conclude that methods, as claimed in the subject application, were conceived prior to October 1996. Further, due diligence was exercised from this time period until the invention was either actually reduced to practice, or until the filing of the subject application.

4. Prior to October 1996, we conceived the invention described in the subject application, i.e., that an immune response could be elicited or enhanced against self tumor antigens by immunization with homologous foreign proteins or portions thereof. In one embodiment of our invention, the self tumor antigen is prostatic acid phosphatase ("PAP"). Experiments from our laboratory confirmed our inventive concept by showing that immunization of rats with human PAP induced an immune response to rat PAP, whereas immunization of rats with rat PAP did not induce an immune response.

5. The following Exhibit (annexed hereto) represents a photocopy of laboratory notebook pages and a Western blot (all from prior to October 1996) kept in the regular course of business at the University of Washington. The dates have been removed from the copies submitted herewith. It our understanding, based on discussion with applicants' representatives, that this is a permissible Patent Office practice.

6. The Exhibit, which is a photocopy of several pages from laboratory notebooks and a Western blot (all from our laboratory), discloses the immunization of Fischer rats ("K") and Lewis rats ("CH") with PAP, e.g., rat PAP ("rPAP") or human PAP ("hPAP"). The Western blot shows that immunization of rats with hPAP elicited antibodies to both human and rat PAP.

7. Included with the Exhibit is a compilation of data from additional experiments (also all prior to October 1996) from our laboratory. The data validates that rat PAP did not elicit an immune response in rats, but immunization of rats with human PAP induced an immune response to both rat and human PAP. Moreover, following immunization of rats with human PAP, the immunized rats could now be boosted with rat PAP.

8. In summary, upon review of our laboratory records, of which the above-cited pages are representative, we have concluded that, at least prior to October 1996, we had conceived of the methods as described and claimed within the subject application. Furthermore, our conception of the invention led to further research, diligently undertaken, resulting in an actual reduction to practice or in the filing of the subject application.

9. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Martin A. Cheever

Dated this 5th day of April, 2002

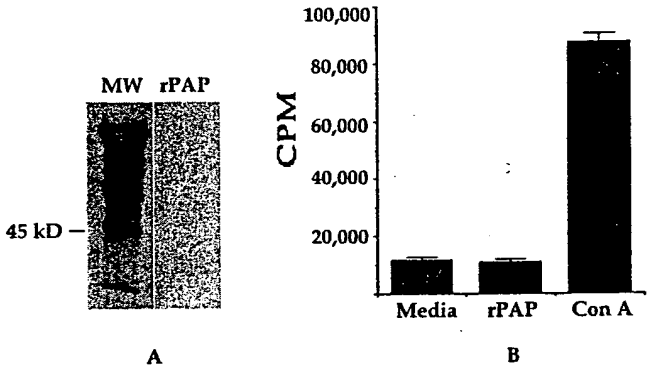
Martin A. Cheever

Mary L. Disis

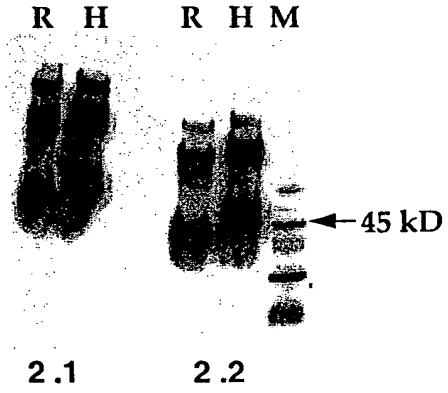
Dated this 14 day of April, 2002

Mary L. Disis

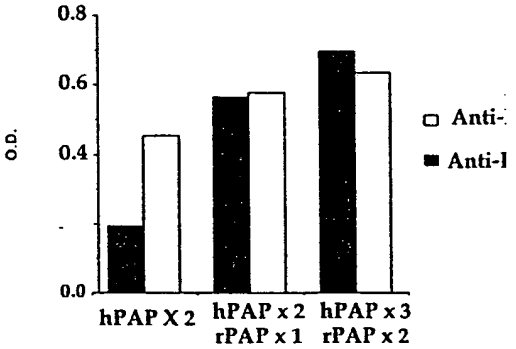
MillicentS/271275



No immunity to rat PAP was induced by immunization with whole rat PAP. Female Lewis rat were immunized with recombinant rat PAP (100 μg) admixed with CFA and boosted twice with rat PAP plus IFA at three-week intervals. The antibody response against rat PAP (rPAP) was determined by western blot analysis (A) as described in Fig. 1. The T cell response was determined by a standard proliferation assay (B). Histapaque-purified splenic mononclear cells (5 x10^5/ml) were incubated with either media, rat PAP (200 μg/ml), or Con-A (5 μg/ml) for 96 hours. Tritiated thymidine (1 uCi/well) was added to the culture for the last 8 hours. The thymidine uptake by T cells was determined by liquid scintillation counting (cpm).



IgG antibody responses to rat PAP could be induced by sequential immunization with hPAP and rPAP. Female Lewis rats were immunized with human PAP (100 μg) plus CFA. They were boosted at three-week intervals with IFA plus human PAP (100 μg) and with IFA plus rat PAP (100 μg). Antibody responses to rat PAP or human PAP were determined by western blot analysis. Rat PAP (R) and human PAP (H) were run on a 10-15 % SDS-PAGE gel under reducing conditions. The blot was incubated with immune sera from two representative animals (2.1 and 2.2), followed by HRP-labeled goat anti-rat IgG antibody. The blot was developed with the ECL detection system. The molecular weight markers (M) are shown on the right.



IgG antibody responses to rat PAP after immunization with human PAP can be significantly boosted by subsequent injections with rat PAP. Female Lewis rats were immunized with human PAP (100 μg) plus CFA. They were boosted sequentially at 3-week intervals with IFA plus human and rat PAP (hPAP x2, rPAP x1); or IFA plus human and rat PAP (hPAP x3, rPAP x2). Sera were obtained two weeks after each boosting. The amount of antibody to rat PAP (solid bars) or human PAP (open bars) in the immune sera was determined by an ELISA assay with 96-well plates pre-coated with either rat PAP or human PAP, respectively. The ELISA assay was developed with HRP-labeled goat anti-rat IgG antibody followed by HRP substrates, and the absorbance at 450 nm was determined.

Rk. G.

(1) ELISA's (Compet. V. —)

100 µg 1:1200 | 1:800 40%
1:1600
1:2000 | 1:1000 57%

100/µg

(2) CH IV 1 Rd ♀ ~~Fl~~
1 Rd ♂ → Imm

(4) Plates →

~~(3) Purification~~

Wednesday

Bone
↓ PDMSF

KI & KIII → (↓ cells)] Cell

KIII

KV → ↓ Absorption

2. Cut Rat X-
7. Spin & Suspend in
8. 0.5% Tri-X / PBS

+ 1ml of Bradford reagent
+ 100λ of sample + H_2O

98λ 96λ 97λ 9[illegible]
Mouse Rat
2λ + 4λ 1λ 2λ

100λ of H_2O as Blank

	595λ		
Blank	0.00		
Mouse 2λ	0.257	→ 1.95mg/ml	1.9 mg/ml
Mouse 4λ	0.474	→ 1.8mg/ml	
Rat 1λ	0.447	→ 6.8mg/ml	8mg/ml
2λ	0.679	→ 1.	
	0.2λ 1.115	→ 8.8mg/ml	

.066 = 1μg

.28 4mg/ml

CTRL Sera — Pre Immune

CH II

Serum & Boost] CH II Tues

Lewis

Serum Boost or CFA] CH III Fri

Swine

If CH II + CH III Are

Spleen cells + Ag + IL-2

→ Spleens

* Rest No

10% Buffer

CTRL

p39

Human

♀ - ♂

Nicely 16 Key

Thursday

* KT Leu & Spleen

* Boost on Tues CH II + CH III

KV

Serve & Sacrifice
GR
P45 (2)
Bovin (4)
(OR)
Freeze everything

— X —

(KV) Our boy

(CHI) CTRL
Hw
p39] Sera Tcell Adoptive Transf

p45
p43

p45 — (A) 140 .1 .2 .3 3 flasks
— (B) 140 .1 .2 .3 3 flasks

(CHI) CTRL
Hw
p39] (Sera + Boost) Sera at sacrifice Boost on sacrifice on.

Gut p45] Sera at sacrifice Freeze spleens

(Rick will run individuals)

Image ~

CHI

① → CTRL	x 2	1	
② → Human PAP	x 4	2	
③ → 39	x 6	2	
④ → 45	x 6	2	
⑤ → 43	x 2	→	

All [illegible]

1st Imm

2nd Imm

3rd Imm

Seen on
!
[illegible] Weiferen's

p39 6 < 3 p39 / 3 [illegible]

p45 6 < [illegible] / 3 [illegible] [illegible]

Peptides 750 µg each   Bovine PAP 400 µg

Kv

① CIRL → 2   1st Imm
② Bovine PAP → 4
③ p39 → 6 — p39 + Rat PAP (Boost)
④ p45 → 6 — [illegible] Rat PAP
⑤ p43 → 2

TODO

* Sera
+ Boost
+ Do Sera from [illegible] on Western

3rd Imm

200μg each   Imm on

Bovine	RaV	Human
100	100	100
100	100	100

KIV   2rats/group

(1) → CTRL
(2) → Bovine + RaV
(3) → Bovine
(4) → Human + RaV
(5) → Human

1 RaV/group remains

1st Imm →
2nd Imm →   } 1 rat/group sacrificed
3rd Imm →
4th Imm →   > 4 wks

TODO

* SACRIFICE OTHER ONE ~ Imm over + COLLECT SERA   IMM and
* √ T cell results (Single 1 rat)
* WESTERNS

	1 rat		Rat 2			
(1)	✓	✓	✓	✓	✓	✓
(2)	✓	✓	✓	✓	✓	✓
(3)	✓	✓	✓	✓	✓	✓
(4)	✓	✓	✓	✓	✓	✓
(5)	✓	✓	✓	✓	✓	✓
	10 gels		20 gels			

KIII

4 + 1 → 2 rats, → Solo, groups

V

① → GM CSF ? ✗

② → Bovine PAP + GM → ✓

③ → Bovine PAP

④ → GM + Rat PAP

⑤ → Rat PAP only (4 Imm through)

To Do

* ✓ T cell responses
* All rats sacrificed on
* Watching

9 gels

~~Peptides~~ ~~Studies~~ Imm Peptides 39 ✓
43 ✓
44 ✓ 25 µg/pt
45 ✓
Protein 400µg total

(K II) Recd 1st Imm c IFA accidentally
2/group

5 groups
- CTRL
- p39 + rPAP
- p43
- p44
- p45 + rat PAP

2 sites SC + 2 sites IM

1st Imm
2nd Imm

Peptides + Protein [400µg total]

(To Do)

* Immunize ✓
- Western ✓ ELISA

✓ Sera collect
— SERA on

✓

K1 — 6 Groups — 1rat/group — 4th Jun

① → PBS/LPA / IFA ⟹ 4 Jun all SQ

② → p39 + 100μg/ml PAP

③ → p43

④ → p44

⑤ → N+S + 100μg/ml PAP

⑥ → ~~Ear~~ PAP → Studied in K3

All rats ✗ Group 6 → SACRIFICED

To do

* ✓ Tcell results

* Wes Results GR 1 — 6 [6 gels

HMW
Human
Homogenate
Ref
1
LMW

1:1

HMW
Human
Homogenate
Ref
1
LMW

2.1 & 2.2

1:1

5 sets